Title: **Production of Native Plants in Potting Media Amended with Flushed Cattle Biosolids**

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FINAL STATUS OF THE PROJECT

Abstract

As a waste product that has plagued the beef and dairy operations for decades with the problem of environmentally sound disposal, cattle biosolids have potential for being an economical and sustainable potting mix amendment, both as a medium substrate and a fertilizer amendment in nursery container production. In this study, we added aged cattle biosolids to a bark and sand mixture in 0, 15, 30, 45, or 60% concentrations (by volume) to make five potting mixes. We planted four species of native perennials—*Holodiscus discolor* (oceanspray), *Philadelphus lewisii* (syringa/mockorange), *Eriogonum niveum* (snow buckwheat), and *Penstemon strictus* (Rocky Mt. penstemon)—into the various mixes and grew them for four months in 1-gallon containers in field blocks. Initial physical and chemical properties of the media were analyzed including aeration, water-holding capacity, total porosity, bulk density, media shrinkage, C:N ratio, electrical conductivity, pH, cation exchange capacity, and mineral concentrations including nitrate-N, ammonium-N, available P, available K, B, S, and extractable K, Ca, Mg, Na, and Cl. All plant heights and penstemon widths were measured every three weeks. When plants stopped growing after two months from low nutrient availability in the mixes, we began weekly supplemental fertilization. At the end of the experiment plant leaves from 60 plants per species were collected for foliar mineral analysis, and the plants themselves were harvested for biomass to compare growth among the mixes. Media physical properties were good for all mixes containing biosolids. Most chemical properties improved with increasing biosolids concentrations. Electrical conductivity was too high ($5.1<EC<7.8$ dSm^{-1}) in media containing ≥30% biosolids by volume, however, and some plants planted into 45 or 60% biosolids amended media suffered salt damage. The biosolids proved to be a nutrient source with mineral concentrations increasing, for the most part, with increasing percentages of biosolids. Over all, plant growth, as determined by height and width measurements and plant shoot and root biomass, improved with increasing biosolids concentration up to 45% biosolids by volume.

Aged cattle biosolids appeared to have some potential for a stable potting media amendment that can improve a bark-based media and provide an additional fertilizer source to native plants grown in amended media.

Materials and Methods:

The experiment, producing native plants in potting media amended with flushed cattle biosolids, was started on May 7, 2007. Unprocessed cattle manure, aged in a waste pile 9 to 12 months, was substituted for flushed biosolids since this latter waste product was unavailable for starting the experiment. Five potting mixes containing either 0, 15, 30, 45, or 60% biosolids (by volume), 10% sand, and 90, 75, 60, 45, and 30% bark, respectively (to bring the volume of each mix to 100%), were thoroughly mixed and supplemented with MicroMax (micronutrient) fertilizer at 1.5 lbs. per cubic yard. The control, 0% biosolids mix, had a pH of 3.74 and required the addition of ground agricultural limestone at 2.25 lbs. per cubic yard. On May 10, 10-cubic inch plugs of the native species *Holodiscus discolor* (oceanspray), *Philadelphus lewisii* (syringa/mockorange), *Eriogonum niveum* (snow buckwheat), and *Penstemon strictus* (Rocky Mt. penstemon) were planted into 1-gallon pots. The plants were established for four weeks before being placed in a randomized complete block design (5 replicates per block x 4 blocks x 5 media x 4 species) on a mulch bed starting June 5. Four hundred plants were used in the study with 100 plants per species and 20 plants of each species per medium.

All mixes were tested for initial aeration, water-holding capacity, total porosity, and bulk density. Media shrinkage was determined by measuring the net change in volume for all pots from the beginning of the experiment until harvest (17 weeks). Initial electrical conductivity (EC) and pH measurements were taken in the field before planting by using the saturated extraction method. The mixes were laboratory tested for initial pH, electrical conductivity, CEC, C:N ratio, soluble salts, and available phosphorus, potassium, nitrate-N, ammonia-N, and sulfur. Later, chloride levels were also analyzed. In early August, pH and EC measurements were taken by pour-through extraction from four randomly selected pots per mix per species.

Since soluble salt levels were high ($5.1<EC<7.8$ dSm^{-1}) in media containing ≥30% biosolids, plants were not fertilized as originally planned in order to compare the fertilizer value of the different percentages of biosolids. After we determined the plants grew little over a 3-week period, all plants in all treatments were fertilized on July 13 with Peters® 30-10-10 (liquid) fertilizer at 100 ppm N. A follow-up application of 15-9-12 Scott's Osmocote® control release fertilizer was added at 12 grams per plant for all treatments on July 16. Plants were then fertilized weekly with 30-10-10 until September 1. Plants were watered with an automated micro-emitter irrigation system fitted with spray stakes. Plants were irrigated every other day, and watering was increased to every day during hot weather to cool the potting medium. The snow buckwheat was removed from automatic watering July 10 and watered only during fertilization due to root sensitivity to media saturation. Toward the first of August, the water to the oceanspray was also cut back to four days a week due to plant stress symptoms from the saturated media, and the snow buckwheat was reset to receive water twice a week to combat wilting brought on by the heat and an early summer growth flush.

Height measurements were taken for all plants and width measurements for penstemon every three weeks beginning June 18 until harvest. At harvest, final heights for all plants and final widths for penstemon and snow buckwheat were taken. Since the biosolids percentage appeared to have some influence on weed presence in the media, weeds were collected every three weeks beginning June 20 and their biomass recorded by treatment. Beginning September 14, 2007, all plants were destructively harvested for tissue analysis and biomass. Leaves from three randomly selected plants per treatment per block were collected for foliar analysis. Five to eight leaves were removed from most stems on the plants. Usually the three upper-most leaves on the stems were excluded, as were the basal leaves, from those used for foliar analysis. Leaves were rinsed with tap water, then rinsed with distilled water for approximately 5 seconds for each sample, and finally dried in a drying oven at approximately 43°C for approximately 10 days until thoroughly dry. The dried leaves were then weighed, ground, and sent to a laboratory to be analyzed for N, P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, B, Na, and Cl. The plants themselves were severed at the root crown, and shoots were dried in a drying oven at 43°C for 21 days until dry then weighed for biomass. To obtain a rough idea of the influence of biosolids percentages on root growth, roots were collected from two species: *Holodiscus discolor* and *Eriogonum niveum.* Root systems from two randomly selected plants from each treatment per block per species were washed free from the medium, and the wash water filtered through 2 mm grain sieves to collect as much of the root material as possible. Roots were then dried in a drying oven at 43°C for 21 days until dry and weighed for biomass.

After final data collection, media chemical and physical properties, plant growth (height and widths), foliar mineral data, and final biomass for shoots and roots were statistically analyzed for treatment significance by analysis of variance procedures. Tests for normality of the data were determined by residual and normality plots and univariate procedures. Fisher's Least Significant Difference (LSD) test was used at the 5% level to determine significant differences between means. If a plant died differences between means for growth parameters were determined by least square mean comparisons at the 5% level.

Results and Discussion:

Initial physical and chemical properties of the media were, for the most part, related to the concentration of biosolids in the potting mixes. Aeration capacity was highly significant across potting mixes, decreasing with the increase from 0% to 60% of biosolids volume (Table 1). Water-holding capacity was inversely related to air capacity, increasing by at least 56% with increasing biosolids percentages, as did bulk density (which increased 68% compared to the mix without biosolids); but total porosity was similar for all media (average of 75.1% across all mixes). The increased level of water-holding capacity could have allowed for less frequent irrigation of media containing over 30% biosolids by volume, but to maintain consistency across the experiment, water was applied in similar volumes at the same time across treatments. Media shrinkage was non-significant, for all treatments (data not shown). The biosolids material most likely decomposed only to a minimal extent, leading to minor decreases in volume over-time or soil contaminants in the aged manure may have stabilized the material. Root growth also played a critical role by actually increasing root ball volume, resulting in "negative" shrinkage.

The concentrations of biosolids in the mixes strongly influenced their initial chemical properties (Table 2). Initial C:N ratio was highest in media without biosolids and decreased with increasing biosolids concentration. Initial cation exchange capacity (CEC) of the media lacking biosolids (controls) was significantly higher than that of the media that contained aged cattle biosolids. Initial EC and pH increased almost linearly with increasing biosolids percentages. Soluble salt levels (as indicated by EC readings) were high in media containing biosolids. Electrical conductivity of the 30%, 45%, and 60% biosolids mixes was well above the threshold for soluble salt toxicity for even salt tolerant species (Table 2). Plants in all media were heavily watered the first week. Salts, however, still caused a problem and damaged some plants in the 45 or 60% biosolids amended media by May 30. Symptoms of salt damage seemed to decline around mid-June; but only after four Oceanspray plants died, and one was heavily damaged. The other species showed only minor chlorosis and recovered well following heavy irrigation. Plants in the 15% and 30% biosolids mixes had excellent initial plant growth with only minor chlorosis on the plants in the 30% biosolids medium.

The concentrations of biosolids in the mixes also strongly influenced their initial mineral concentrations (Table 3). Except for ammonium nitrogen, nutrient concentrations increased with increasing biosolids percentages in the potting mixes. Aged manure provided a source of nitrate, available potassium, available phosphorus, boron, and sulfur. The manure was also a fairly good source of extractable nutrient cations K, Mg, and Ca—which includes both nutrients in solution and at cation exchange sites (Table 4). These mineral levels were high enough, however, to provide an initial reservoir for plant use. While sodium levels were higher in the media containing higher biosolids percentages, they were too low to be the source of the salt toxicity observed in plants in the 45% and 60% treatments. Extractable chloride was high in all mixes containing biosolids (Table 4) and was probably one source of the salt problems observed early in the experiment. Since chloride is readily leached through media substrate, its loss by leaching would also explain the eventual recovery of mildly affected plants after increased frequency of irrigation. Due to low nutrient content, nutrient deficiency symptoms were visible on plants in the mix without biosolids within a week after being planted, and plants in this medium failed to grow much during the first month of the experiment (Figures 3 through 7).

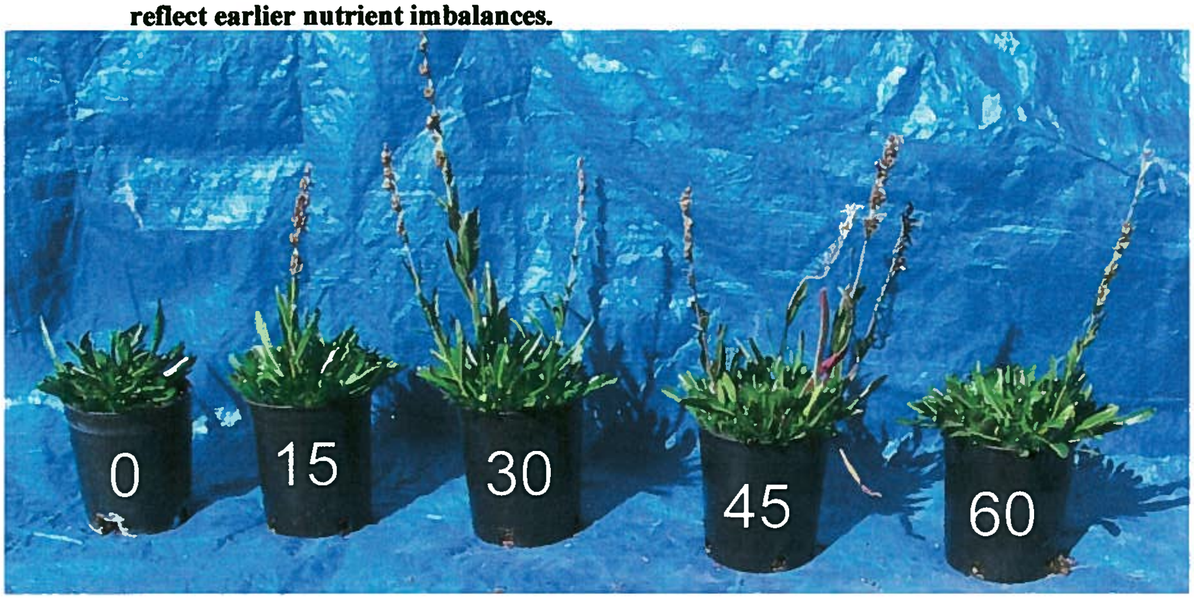
Nutrient deficiency became evident for all plants around July 2 regardless of biosolids percentages in the potting mixs. Plant heights and widths measured every 3 weeks increased very little from June 18 to July 9 (Figures 3 through 7). As a result, fertilization was begun on July 13. A quick field test by pour-through extraction for EC and pH conducted August 8 provided some reasons for the increasing nutrient deficiency. Electrical conductivity was very low, indicating nutrient deficiency within the media, while pH levels had increased by a full unit and were high enough (data not shown) to reduce micronutrient availability in the media. The decrease in EC over time was due to the daily irrigation that constantly leached ions from the media, also lowering nutrient content from the biosolids amendment.

More weeds grew in mixes with higher percentages of biosolids than in those with lower percentages (data not shown). Most weeds grew during cooler weather immediately after planting or again in early fall before harvest. Weed species present biosolids-amended media included common lamb's quarters, red root pigweed, common groundsel, and common mallow. The weed growth in the media containing manure would necessitate herbicide application were aged cattle biosolids to be used in a commercial nursery setting.

The relationship between plant growth and biosolids percentage was apparent by mid-June. In spite of initial salt damage to plants in the 45% and 60% biosolids potting mixes, the manure did provided a fertilizer boost compared to media without biosolids (Figures 3 through 7). Plants grown in media without biosolids (controls) failed to grow after establishment in the field (Figure 1) until supplemental fertilization was begun. Once fertilization began, plants in this mix grew rapidly and by the end of the experiment had caught up to the final heights and widths of the plants grown in media containing biosolids (Figures 2 and 3 through 7). Penstemon plants in the 0% and 60% biosolids treatments grew the most over time, reflecting the recovery of the plants in the 0% mix from nutrient deficiency and in the 60% mix from the salt stress at the beginning of the experiment (Figures 1, 2, 6 and 7). Plants in all media responded strongly to supplemental fertilization following the second height measurement (compare Figures 1 and 2 and 3 through 7). This response was strongest for the mockorange and the penstemon (Figures 3, 6, and 7), indicating these species are capable of using higher quantities of nutrients and better at adapting their usage to nutrient availability. The mockorange, in particular, grew rapidly and continued to grow up to the final height measurement (Figure 3). Penstemon planted in media lacking biosolids responded the most, resulting in similar overall growth toward the end of the experiment. Both snow buckwheat and oceanspray were less responsive to supplemental fertilization (Figures 4 and 5, respectively), which would indicate a lower nutrient requirement by the plants. The higher incidence of salt damage to the oceanspray supports this species preference for lower nutrient concentrations in the medium.

Figure 1. Penstemon plants in a field block on June 18, 2007, showing fertilizer effects from increasing biosolids pernetages. This block is sequenced 0%, 15%, 30%, 45%, and 60% biosolids concentrations in potting mixes in each row from left to right in the picture. In the row far left, plants in the 0% treatment showed nutrient deficiency symptoms, and plants in the row far right (the 60% treatment) showed some salt damage.

Figure 2. Penstemon plants photographed at harvest (September 24). The plants were arranged from the left by biosolids concentrations in the potting mixes (by volume). Plant growth in the differing media was similar for plants in different media by the end of the study due to supplemental fertilization, except for the relative number of flower spikes which reflect earlier nutrient imbalances.



The percentage of biosolids in the media affected the plant biomass of all species. Shoot biomass for both mockorange and penstemon increased linearly with increasing biosolids concentrations (Figures 9 and 12). Oceanspray and snow buckwheat increased in shoot biomass for plants in media with 30 to 45% biosolids concentrations before shoot biomass began to decline in the 60% biosolids treatment (Figures 10 and 11). The decline was probably the result of salt sensitivity and salt damage early in the growing season as indicated by the extremely high electrical conductivity in the media (Table 2). Root biomass also increased significantly for oceanspray and snow buckwheat, as the percentages of biosolids increased up to 30 or 45% before causing a decrease in root growth (data not shown).

Although higher percentages of biosolids increased nutrient concentrations in the potting mixes, biosolids concentration affected foliar plant nutrients only to a minor extent. Phosphorus, magnesium, calcium, manganese, and boron foliar concentrations were significant for almost all the species analyzed. In mockorange leaves, P, Ca, and B tended to be most concentrated in foliage from plants grown in media containing 30% biosolids, showing that while the manure was a potential mineral source, media chemistry at higher biosolids percentages may have inhibited mineral uptake. Magnesium and manganese were more available to the plants grown in mixes with lower biosolids concentrations, indicating that bark was the best source of these nutrients for the mockorange. In spite of statistical significance of phosphorus, calcium, and boron, these mineral concentrations in plant foliage appeared unaffected by biosolids concentrations. Small differences in foliar mineral concentrations most likely lack biological significance, even though certain mineral concentrations were statistically different among plants grown in the various media. Supplemental fertilization may have altered foliar nutrition, since species in all mixes received the same rate of application. By the time the foliar tissue was harvested, foliar nutrient content most likely became similar across treatments.

While little is known about the foliar mineral concentrations needed by the particular species, the range of most nutrients analyzed was probably sufficient in the experiment. For example a domestic cultivar of mockorange has higher foliar concentration of N, K, Ca, Mg, Cu, Mn, and B compared to the native species used in this study. However, nutrient requirements would most likely be lower for the non-domesticated mockorange; therefore, these nutrient concentrations may have been sufficient (Tables 5 and 6). For some nutrients, tissue concentrations were actually high (in particular Fe in oceanspray and Zn in snow buckwheat) when compared to requirements on record for known species. The plants themselves appeared healthy, although the oceanspray showed signs of early leaf senescence.

Significance to the Nursery Industry:

Overall, aged cattle biosolids showed potential as both a fertilizer source and substrate amendment for potting media for native plant production. Native plants grown in media containing 30 or 45% biosolids by volume grew well throughout the 4-month experimental period as opposed to plants in media without biosolids (controls), which failed to grow until they received supplemental fertilization. Media containing 30 or 45% biosolids produced the best overall growth across species. Some minor salt stress of plants grown in these media was observed early in the experiment, but heavy irrigation lowered salt levels, and effected plants quickly recovered. Media containing less than 30% biosolids by volume had limited fertility and lower water-holding capacity, which would require more frequent irrigation and earlier applications of supplemental fertilizer in a commercial nursery. The absence of media shrinkage even in those mixes containing the highest percentages of biosolids indicated promise as a physically stable medium amendment. High soluble salt levels as indicated by high EC, resulted in salt damage and some mortality to plants grown in 45 or 60% biosolids-amended media. Some of the salt problem, however, was reduced by leaching the media, and eventually EC was reduced to below even desired levels. An overall increase in pH overtime, probably the result of breakdown of the organic matter from the biosolids, may have contributed to nutrient deficiency and limited nutrient availability over time, reducing the effectiveness of the biosolids as a fertilizer source. Supplemental fertilization offset the problem, for the most part, since foliar mineral analysis failed to reveal any serious deficiencies. Further research would be needed to determine the best overall percentage of biosolids required for optimum yield and plant performance. Further study using a cattle biosolids product, such as flushed or biodigested material, would also be valuable to evaluate a material that would have better consistency both from year to year and from a single source.

Table 1. Initial physical properties of media amended with various percentages (by volume) of aged cattle biosolids.

Biosolids	Air Capacity	Water-holding Capacity	Total Porosity	Bulk Density
(%)		(%)		($g \cdot cm^{-3}$)
0	40.1 a*	35.8 d	75.9	0.299 d
15	27.0 b	46.8 c	73.8	0.404 c
30	25.0 bc	50.4 bc	75.4	0.416 bc
45	24.1 c	50.8 b	74.9	0.431 b
60	19.5 d	55.9 a	75.4	0.505 a

*Means followed by different letters within a column are significantly different at P<0.05 Fisher's LSD test (n = 4).

Table 2. Initial chemical properties of media amended with various percentages (by volume) of aged cattle biosolids.

Biosolids (%)	pH	EC ($dS \cdot m^{-1}$)	CEC ($cmol(+) \cdot kg^{-1}$)	C:N Ratio
0	5.2 e*	1.5 e	24 a	115 a
15	6.0 d	3.3 d	19 b	50 b
30	6.5 c	5.1 c	21 b	32 c
45	6.8 b	6.4 b	20 b	25 cd
60	7.1 a	7.8 a	21 b	20 d

*Means followed by different letters within a column are significantly different at P<0.05 Fisher's LSD test (n = 4).

Table 3. Initial mineral concentrations of media amended with various percentages (by volume) of aged cattle biosolids.

Biosolids	Available K	Available P	Available B	Nitrate N	Ammonium N	Sulfate S
(%)			(ppm)			
0	250 e*	43 e	2.3 d	4.0 e	11.0 a	280 d
15	1430 d	340 d	4.0 c	100.0 d	10.6 ab	330 c
30	2500 c	635 c	4.6 b	237.5 c	8.6 bc	340 bc
45	3530 b	868 b	6.0 a	355.0 b	8.0 c	360 b
60	4350 a	1075 a	6.3 a	507.5 a	6.8 c	400 a

*Means followed by different letters within a column are significantly different at P<0.05 Fisher's LSD test (n = 4).

Table 4. Initial mineral concentrations of extractable ions in media amended with various percentages (by volume) of aged cattle biosolids.

Biosolids	Extractable Ca	Extractable Mg	Extractable K	Extractable Na	Extractable Cl
(%)			(mmol(+)·L^{-1})		
0	14 e*	4.5 d	0.7 e	0.7 e	19 e
15	20 d	6.3 c	3.4 d	1.0 d	115 d
30	31 c	6.9 bc	6.7 c	1.4 c	268 c
45	36 b	7.4 ab	9.7 b	1.6 b	348 b
60	40 a	8.1 a	11.5 a	1.9 a	458 a

*Means followed by different letters within a column are significantly different at $P<0.05$ Fisher's LSD test (n = 4).

Table 5. Mean macronutrient concentrations in leaves from four species of native plants. Foliar nutrient concentrations shown for each species were averaged over all plants in all potting mixes.

	N	P	K	Ca	Mg	S
Species			(%)			
Mockorange	2.10	0.26*	1.46	0.96*	0.28*	0.15
Snow Buckwheat	3.41	0.43	1.53	1.23*	0.67*	0.25
Oceanspray	2.39*	0.26*	1.00*	0.64*	0.35*	0.24*
Rocky Mt. Penstemon	2.55	0.36*	1.66*	1.16*	0.43	0.32

*Asterisks following nutrient level means indicate significance among treatments (n = 60).

Table 6. Mean micronutrient concentrations in leaves from four species of native plants. Foliar nutrient concentrations shown for each species were averaged over all plants in all potting mixes.

	Fe	Zn	Cu	Mn	B	Na	Cl
Species			(ppm)			(%)	
Mockorange	102*	29	5	24*	37*	0.004	0.04
Snow Buckwheat	212	88*	9	35*	66*	0.041	0.17
Oceanspray	371*	35	11	48*	34*	0.043	0.01
Rocky Mt. Penstemon	82	39	6	46*	42*	0.006	0.14*

*Asterisks following nutrient level means indicate significance among treatments (n = 60).

Figure 3. Change in mean heights of mockorange plants grown in potting mixes amended with 0, 15, 30, 45, or 60% aged biosolids by volume. Data points are means of 20 plants. The arrow on the x-axis marks approximately where weekly supplemental fertilization began on July 13. Error bars indicate standard error.

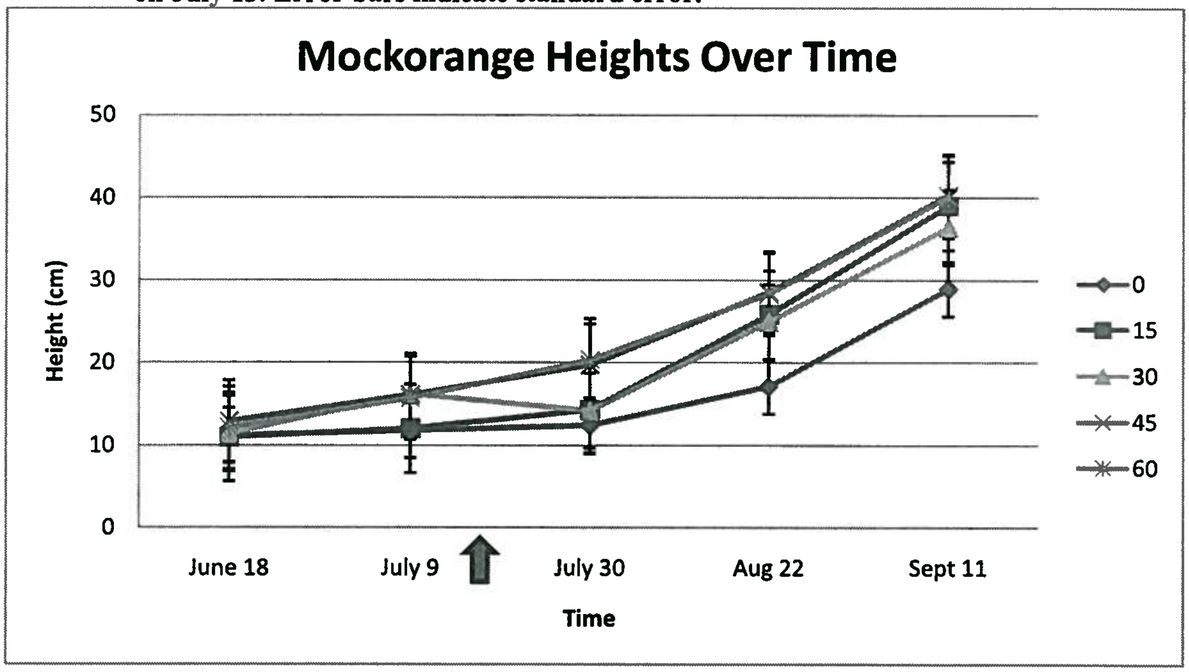


Figure 4. Change in mean heights of snow buckwheat plants grown in potting mixes amended with 0, 15, 30, 45, or 60% aged biosolids by volume. Data points are means of 20 plants. The arrow on the x-axis marks approximately where weekly supplemental fertilization began on July 13. Error bars indicate standard error.

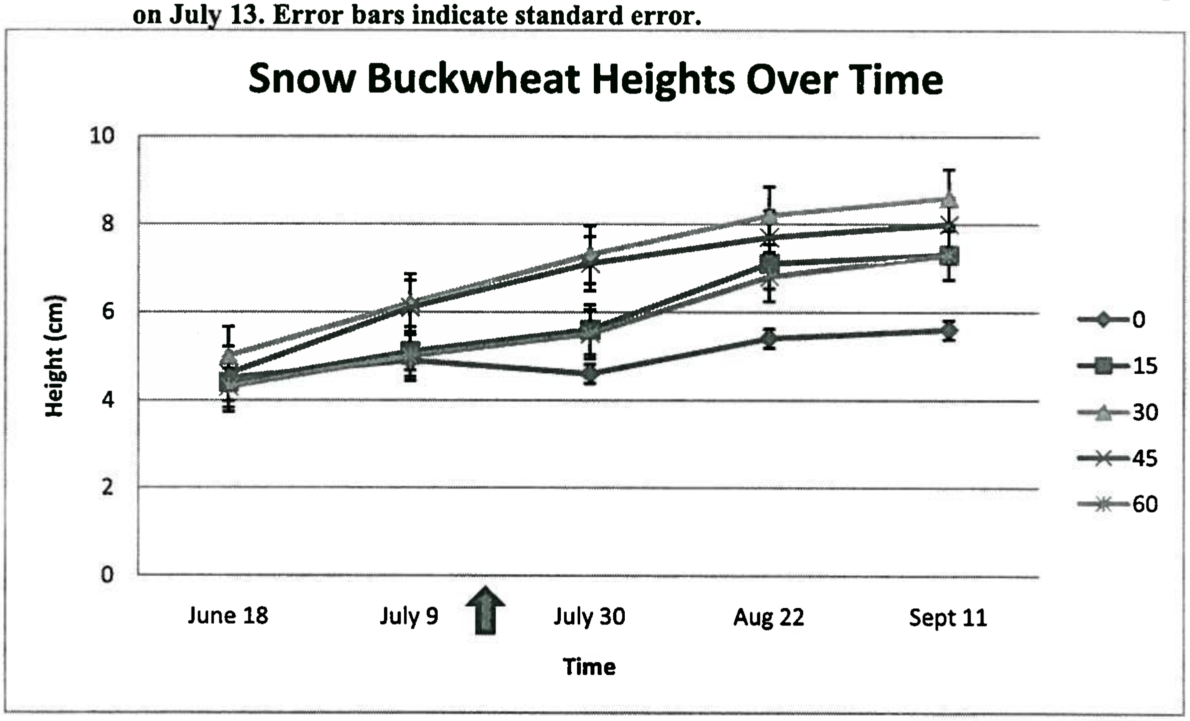


Figure 5. Change in mean heights of oceanspray plants grown in potting mixes amended with 0, 15, 30, 45, or 60% aged biosolids by volume. Data points are means of 20 plants. The arrow on the x-axis marks approximately where weekly supplemental fertilization began on July 13. Error bars indicate standard error.

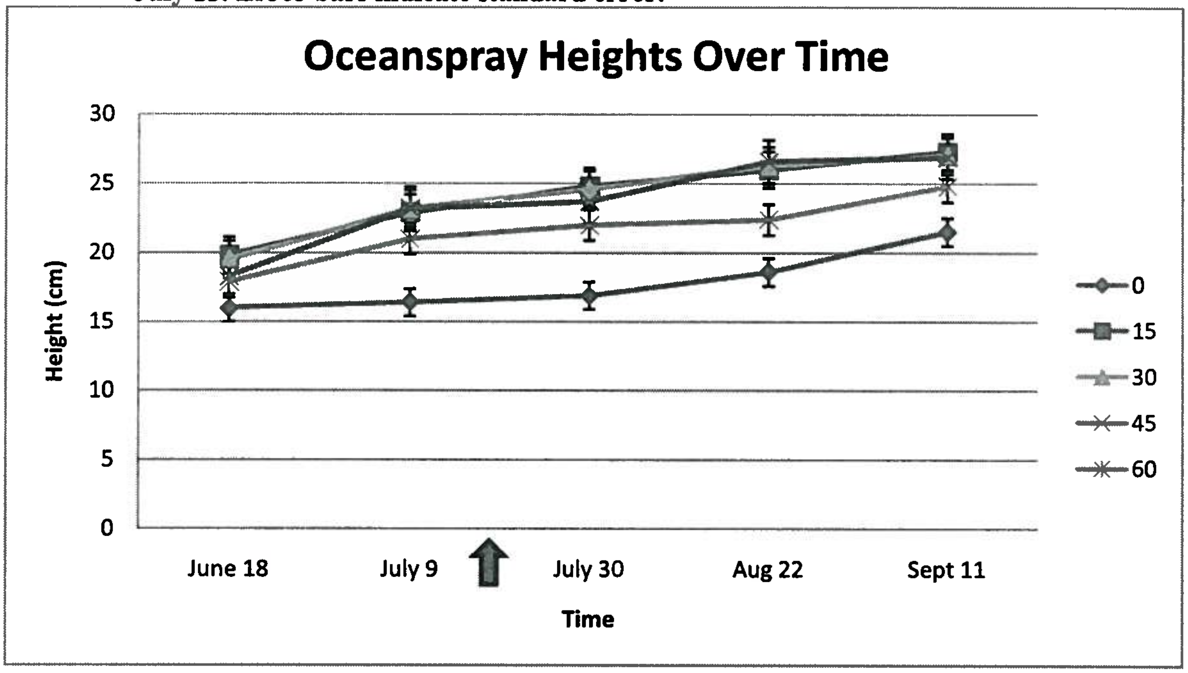


Figure 6. Change in mean heights of Rock Mt. penstemon plants grown in potting mixes amended with 0, 15, 30, 45, or 60% aged biosolids by volume. Data points are means of 20 plants. The arrow on the x-axis marks approximately where weekly supplemental fertilization began on July 13. Error bars indicate standard error.

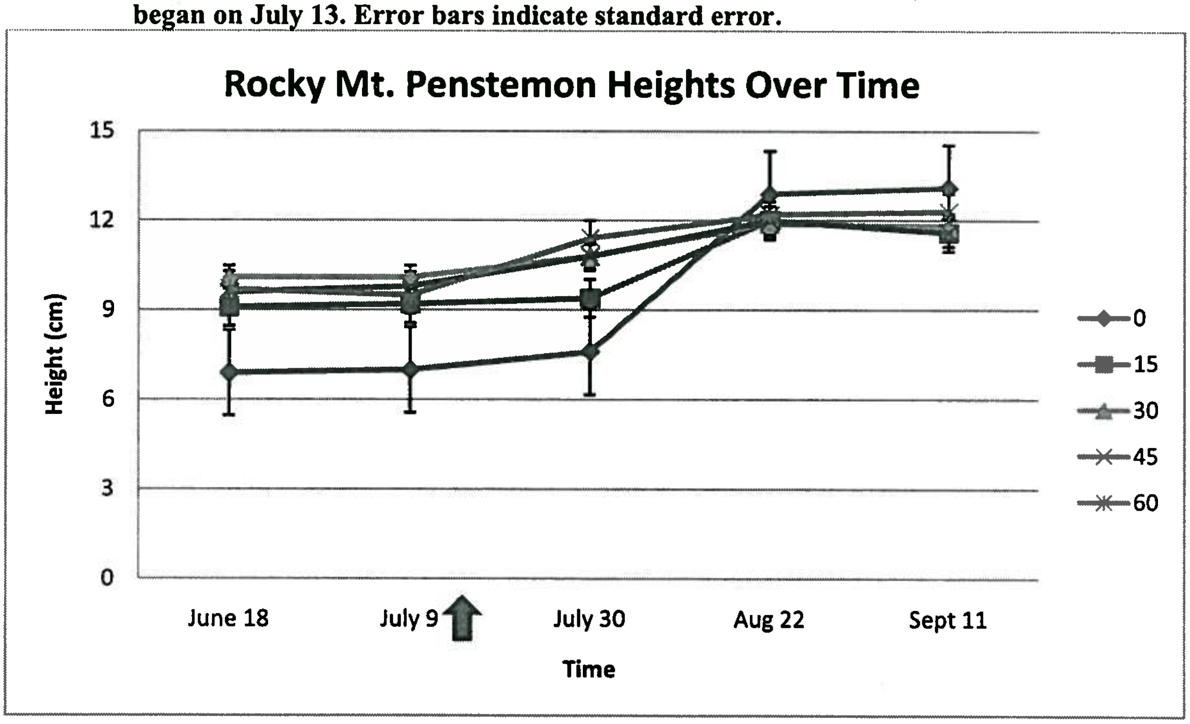


Figure 7. Change in mean widths of Rock Mt. penstemon plants grown in potting mixes amended with 0, 15, 30, 45, or 60% aged biosolids by volume. Data points are means of 20 plants. The arrow on the x-axis marks approximately where weekly supplemental fertilization began on July 13. Error bars indicate standard error.

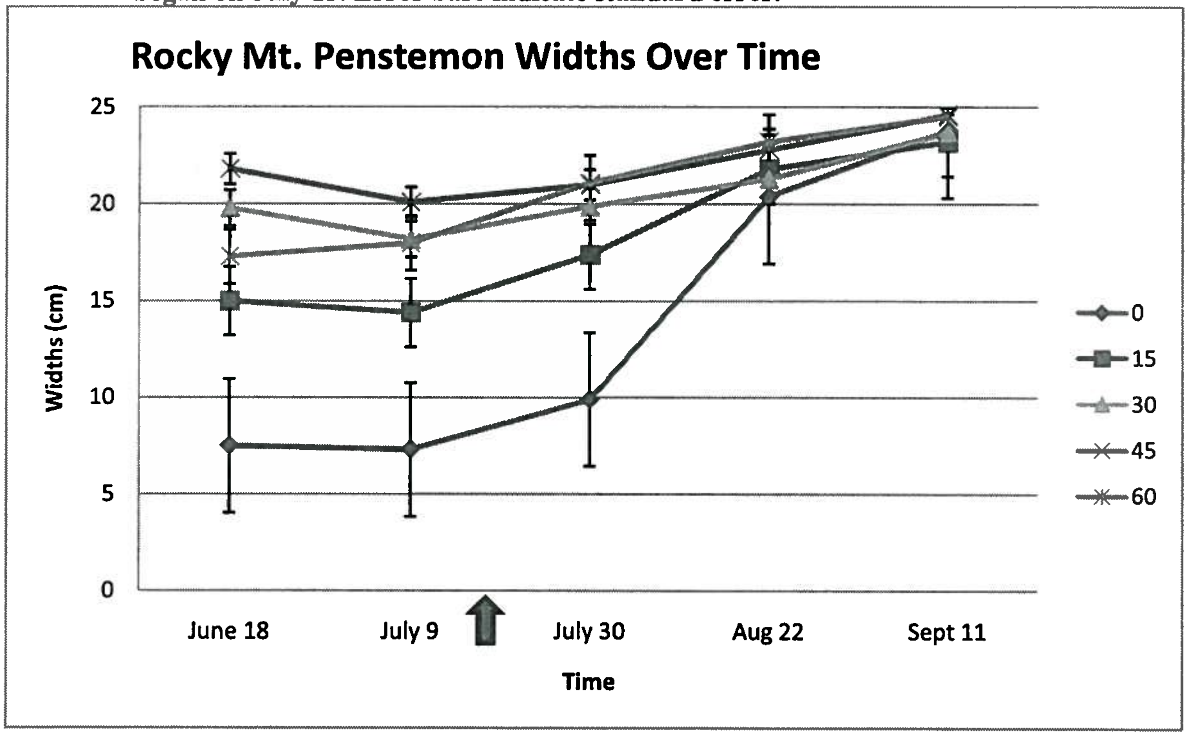


Figure 8. Mean shoot biomass of mockorange plants after growing for four months in potting mixes amended with 0, 15, 30, 45, or 60% aged cattle biosolids by volume. Different letters indicate significant differences as determined by protected Fisher's LSD test at the 5% level (n = 20).

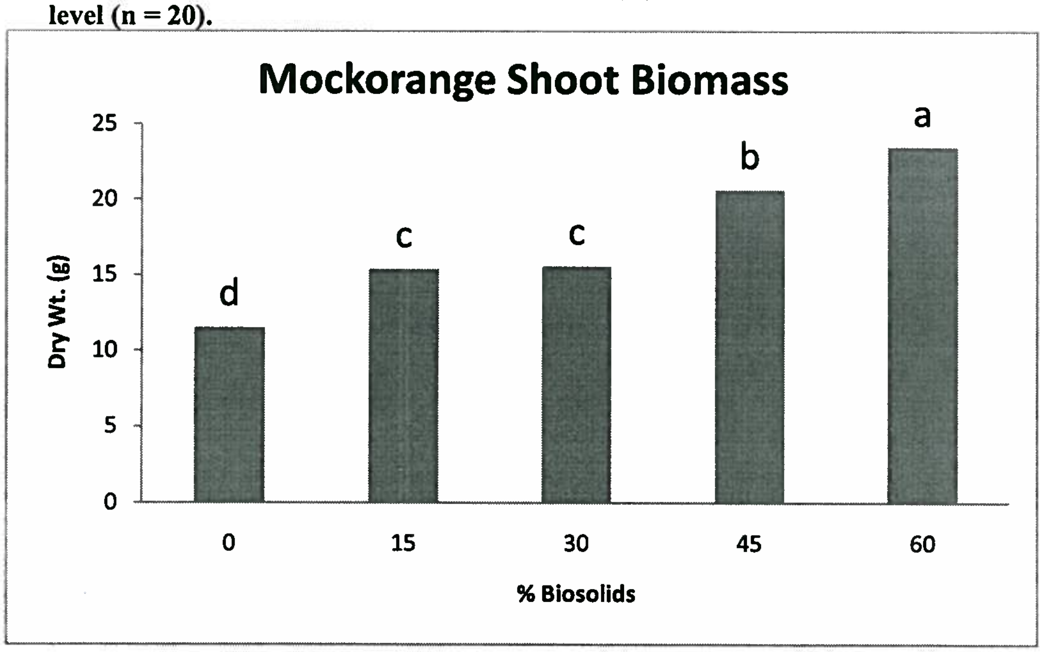


Figure 9. Mean shoot biomass of snow buckwheat plants after growing for four months in potting mixes amended with 0, 15, 30, 45, or 60% aged cattle biosolids by volume. Different letters indicate significant differences as determined by protected Fisher's LSD test at the 5% level (n = 20).

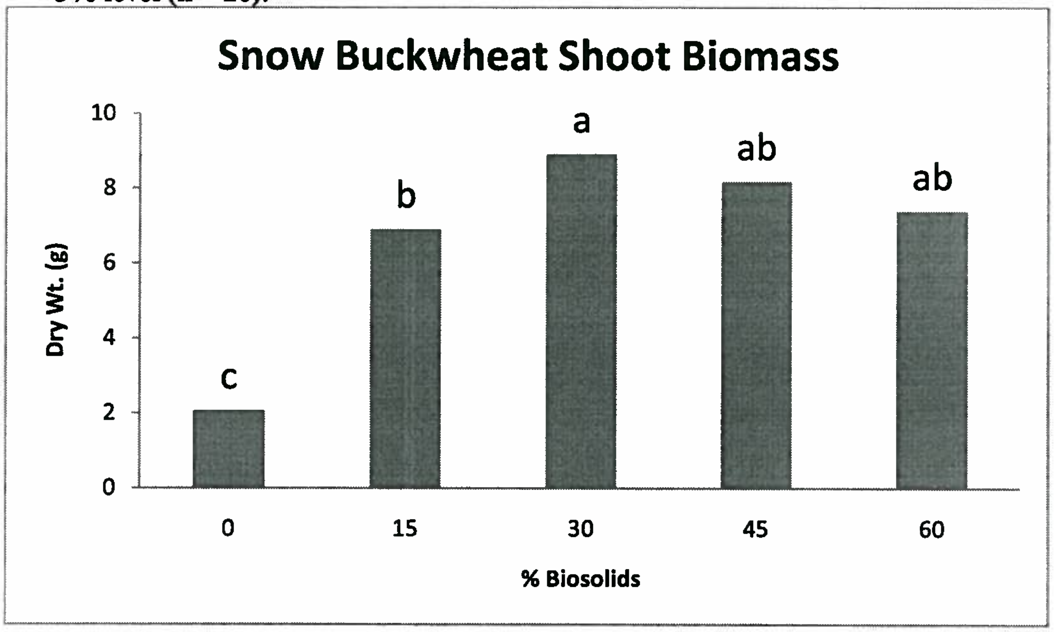


Figure 10. Mean shoot biomass of snow buckwheat plants after growing for four months in potting mixes amended with 0, 15, 30, 45, or 60% aged cattle biosolids by volume. Different letters indicate significant differences as determined by protected Fisher's LSD test at the 5% level (n = 20).

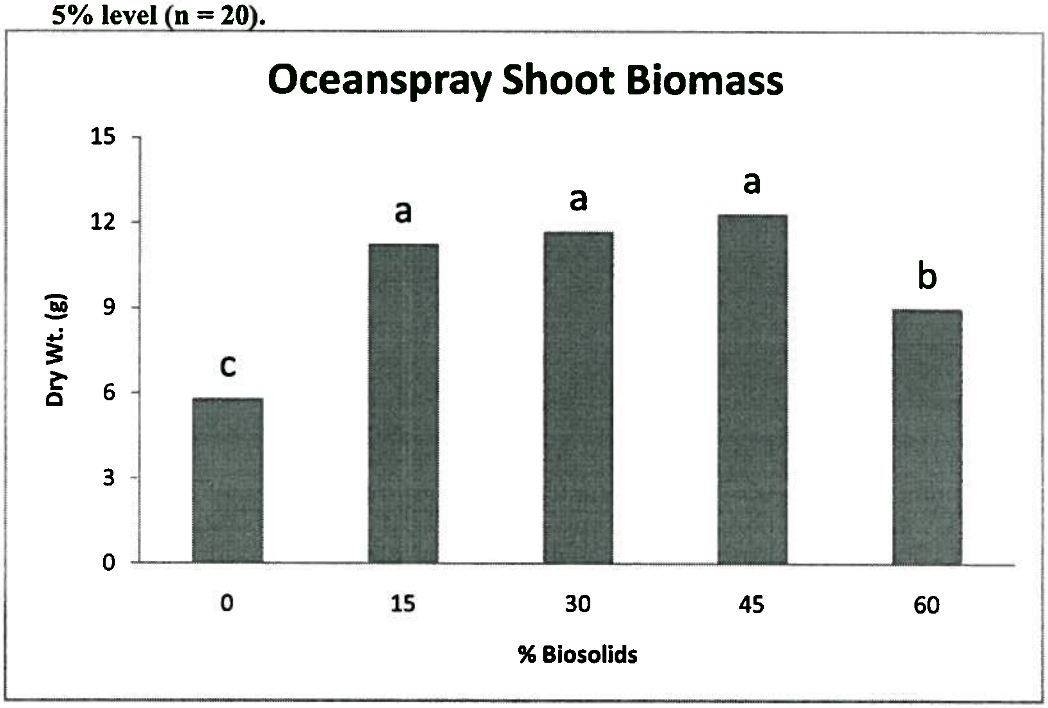


Figure 11. Mean shoot biomass of snow buckwheat plants after growing for four months in potting mixes amended with 0, 15, 30, 45, or 60% aged cattle biosolids by volume. Different letters indicate significant differences as determined by protected Fisher's LSD test at the 5% level (n = 20).